

THE BIOSENTINEL BIOANALYTICAL MICROSYSTEM: CHARACTERIZING DNA RADIATION DAMAGE IN LIVING ORGANISMS BEYOND EARTH ORBIT

A.J. Ricco,¹ R. Hanel,¹ S. Bhattacharya,¹ T. Boone,¹ M. Tan,¹ A. Mousavi,¹ A. Rademacher,¹ A. Schooley,¹ B. Klamm,¹ J. Benton,¹ M. Padgen,¹ D. Gentry,¹ C. Friedericks,¹ G. Defouw,¹ M. Parra,¹ S. Santa Maria,¹ D. Marina,¹ BG Swan,² S. Wheeler,² S. Gavalas,² B. Lewis,¹ H. Sanchez,¹ J. Chartres,¹ and T. Lusby¹

¹NASA Ames Research Center, Moffett Field, California, USA

¹NASA Johnson Space Center, Houston, Texas, USA

ABSTRACT

We will present details and initial lab test results from an integrated bioanalytical microsystem designed to conduct the first biology experiments beyond low Earth orbit (LEO) since Apollo 17 (1972). The 14-kg, 12x24x37-cm BioSentinel spacecraft (Figure 1) assays radiation-responsive yeast in its science payload by measuring DNA double-strand breaks (DSBs) repaired via homologous recombination, a mechanism common to all eukaryotes including humans. *S. cerevisiae* (brewer's yeast) in 288 microwells are provided with nutrient and optically assayed for growth and metabolism via 3-color absorbptiometry monthly during the 18-month mission. BioSentinel is one of several secondary payloads to be deployed by NASA's Exploration Mission 1 (EM-1) launch vehicle into ~0.95 AU heliocentric orbit in July 2018; it will communicate with Earth from up to 100 million km.

MOTIVATION & BACKGROUND

For life to live and thrive beyond low Earth orbit requires understanding and managing multiple unique perturbations. While the International Space Station and other orbiting spacecraft provide reduced-gravity environments, the complex radiation environment of interplanetary space, comprising many particle types, each with its own energy spectrum, is not reproduced by any terrestrial or orbital facility. Full radiation-response characterization necessitates chronic exposure and monitoring of live biology beyond Earth's magnetosphere.

Small autonomous satellites, called nanosatellites or cubesats, simultaneously reduce cost and increase accessibility for space-science experiments [1]. Leveraging and integrating advances in nano-, micro-, and miniature technologies in fields from biotechnology to microfluidics to telecommunications, small satellites are being developed by over 100 universities, numerous small ventures, large aerospace companies, multiple developing nations, and all major space agencies. Despite their diminutive size, they support complex science [1-3]. By coupling autonomy and telemetry in these space "free flyers", near-real-time experimental data are provided from environments that may be challenging or costly for human missions [2,3].

EXPERIMENT, DESIGN, & INITIAL RESULTS

BioSentinel's 'canary in the coal mine' strategy assesses health risk for long-duration human missions beyond LEO using microwell arrays to monitor DNA DSB/repair in three strains of *S. cerevisiae*: wildtype (control); a mutant strain sensitized to radiation; an engineered strain that reports repaired DSBs via growth.

Figure 2 shows one of BioSentinel's 18 microfluidic "minicards", fabricated by precision machining, laser/blade cutting, and lamination with pressure-sensitive adhesive.

"Track-etched" translucent polycarbonate membranes provide crosstalk-free monolithic inlet/outlet filtration, confining yeast to microwells while permitting light transmission measurements.

Figure 3, a cross-section of a single fluidic well, shows the optical measurement and thermal control components. Three surface-mount LEDs provide illumination (570/630/850 nm in sequence); absorbances are calculated from intensity-to-frequency light sensors (ams/TAOS). Minicard cover layers are optical-quality, permeation-resistant poly(cycloolefin).

One 16-well fluidic minicard is activated monthly during the 1.5-year mission to quantify accumulated DNA DSBs and other radiation damage. *S. cerevisiae*, air-dried before launch onto microwell walls, grows upon μ well filling with a 9:1 mixture of growth medium and indicator dye (alamar blue), which changes blue \rightarrow pink \rightarrow colorless due to cellular metabolism [2]. Filling and metering are accomplished by a miniature peristaltic pump (Takasago) and 3-way solenoid valves (Lee).

Figure 4 shows biological growth and metabolism results from lab testing of a flight prototype of the integrated optical/fluidic/thermal/bioanalytical system.

Besides its bioanalytical microsystem, BioSentinel includes two physical radiation sensors (Figure 5) to measure total ionizing dose and linear energy transfer. The latter measures energy deposited by each particle per unit length traversed and thus defines its potential to cause (biological) damage.

CONCLUSIONS

Directly studying biology's response to the interplanetary space radiation environment has been impractical absent costly human beyond-LEO missions. Robust, autonomous bioanalytical microsystems compatible with small satellites are poised to change this. BioSentinel is supported by NASA's Advanced Exploration Systems division.

WORD COUNT: 593

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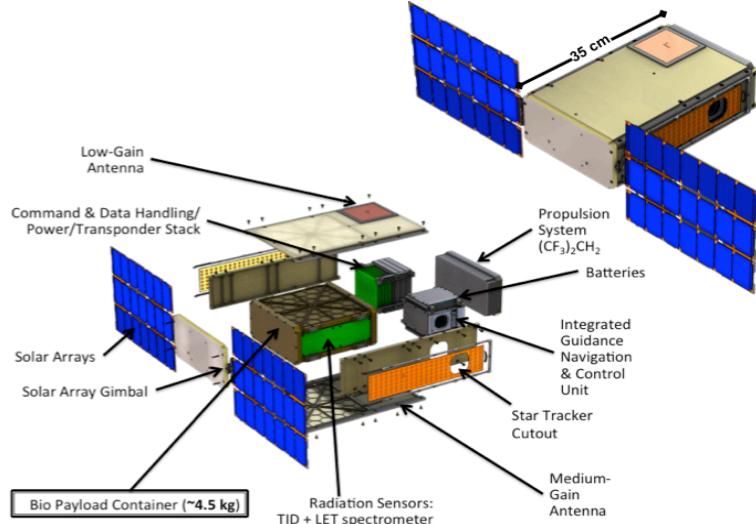


Figure 1. BioSentinel spacecraft views: exploded (left) and assembled (upper right). Solar panels (~ 35 W) are pointed toward the sun by the guidance, navigation & control (GN&C) unit, which includes star tracker. GN&C unit also points choice of radio antenna at Earth.

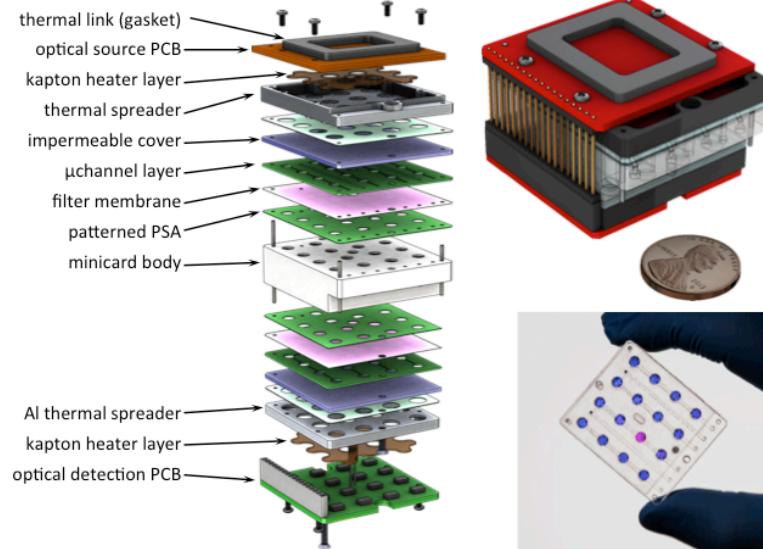


Figure 2. Exploded (left) and assembled (upper right) views of the microfluidic minicard with integral thermal control and optical measurement components. Bottom right: photo of a functional prototype fluidic minicard; 15 wells contain alamar blue and one well contains the same dye that has turned pink due to yeast metabolic activity. (For μ well cross sections, see Fig. 3.)

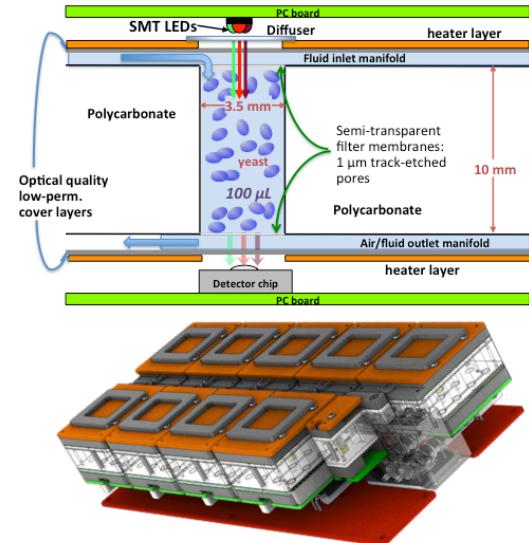


Figure 3. Top: Cross section of one fluidic μ well (100 μ L) with integral filter membranes and connecting microchannels. Dried yeast on walls grow and bud when medium is introduced in spaceflight, displacing air through porous membrane. Three surface-mount LEDs per well, with thin-film diffuser, provide green, red, and near-IR illumination to monitor metabolic activity and track yeast growth; readout uses the detector at bottom of each well. Patterned metal-on-kapton heaters with Al thermal spreaders maintain uniform, stable growth temperature ($\sim 20 \pm 1$ $^{\circ}$ C). Bottom: Nine fluidic minicards will be integrated and manifolded together for flight; two such sets (288 μ wells) will be included in the bio payload container of Fig. 1.

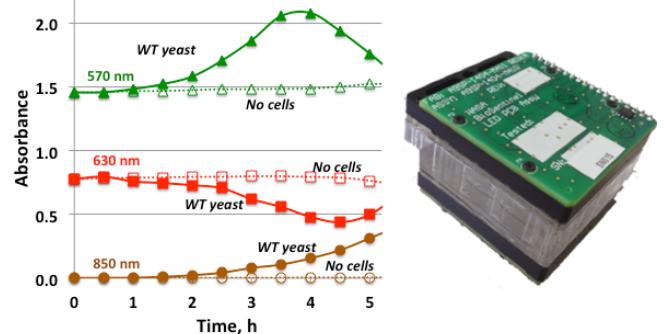


Figure 4. Growth curves (left) recorded using spaceflight optical/fluidic/thermal prototype (right) for wildtype (WT) *S. cerevisiae* with added alamar blue (filled symbols) and cell-free controls (open symbols); LED wavelengths as indicated. Green absorbance increases as pink color appears, then decreases as pink fades. Red absorbance decreases as blue dye turns pink, then increases due to cell population growth. Near-IR absorbance, unaffected by all forms of the dye, tracks cell population.

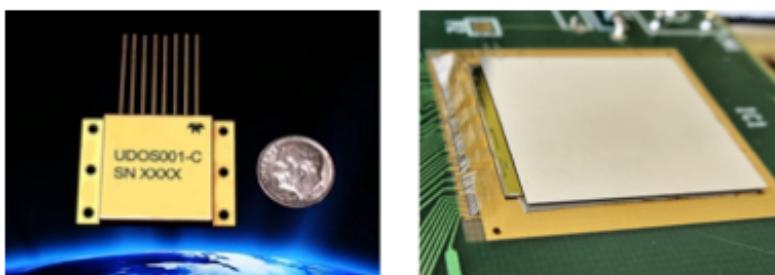


Figure 5. Physical sensors for total ionizing dose (left, Teledyne μ DOS001) and linear energy transfer (center, TimePix chip, being implemented in LET sensor system by Johnson Space Center Radworks Group). Typical data frame (right) from LET sensor shows one spot or track per high-energy particle impact. Analysis of each spot/track provides the associated LET of that event. Count of particle events, binned by LET, will be recorded hourly in space.